Letters to the Editor

Am. J. Hum. Genet. 60:729–730, 1997

Nonsense Mutations and Altered Splice-Site Selection

To the Editor:

The invited editorial by Maquat (1996), regarding defects in RNA splicing and the consequence of shortened translational reading frames, provided a balanced and comprehensive review of the topic. We believe, however, that our work describing the nonsense codon–mediated skipping of fibrillin-1 exon 51 (Dietz et al. 1993; Dietz and Kendzior 1994) was interpreted in a manner that is not fully supported by our data.

Maquat used two arguments to forward the hypothesis that the altered splicing was caused by a change in mRNA structure rather than by a change in the mRNA coding potential. First, she stated that "of the eight reported frameshift and nonsense mutations within the fibrillin gene that reduce mRNA abundance, only the nonsense mutation within exon 51 is accompanied by altered splicing" (p. 283) Second, it was noted that whereas the expression of fibrillin minigenes harboring the exon 51 nonsense mutation resulted in exon skipping, the expression of minigenes in which the nonsense mutation was out of frame reduced, but did not abolish, the abnormal splicing phenotype. A number of issues need to be clarified. To my knowledge few, if any, of the other premature-termination codons (PTCs) described for the fibrillin-1 gene have been comprehensively assayed for induced splicing defects. In addition, it is possible and even likely that the context within which a premature translational stop signal is found can influence the initiation and efficiency of altered splicing. Indeed, context-specified constraints are known to influence nonsense-mediated mRNA decay (NMRD), a process that is clearly dependent on the interruption of coding potential. The best-characterized factor is the nonsense codon's relative position within the gene, with PTCs at the extreme 3′ end being least likely to initiate transcript decay. Both the reason why different nonsense mutations in the same region of the same gene can be associated with variable efficiencies of NMRD and the basis for the complete immunity of some transcripts harboring selected nonsense mutations in central exons remain to be elucidated. Finally, in our fibrillin-1 minigene experiments, we showed that all three nonsense mutations (TAG, TAA, and TGA) at codon 2113 in exon 51 induced exon skipping, whereas a synonymous mutation (TAC) did not. Furthermore, placing the nonsense mutations out of frame with the initiating ATG resulted in complete (TAA or TGA) or significant but partial (TAG) restoration of the normal splicing phenotype (exon inclusion). The fact that the TAG mutation creates a strong cryptic splice acceptor that may compete with the constitutively used acceptor and may impair exon definition, a potential confounding variable, was discussed in our article (Dietz and Kendzior 1994).

It recently has been shown that PTCs can inhibit the splicing of immunoglobulin light-chain pre-mRNAs and that this effect can be recapitulated by use of nuclear extracts devoid of ribosomes (Lozano et al. 1994; Aoufouchi et al. 1996). Taken together, these observations suggest a cellular mechanism that appreciates the coding potential of nuclear pre-mRNAs. The difficulty in reconciling this model with known methods of deciphering reading frame does not obviate its validity but, rather, spurs the search for the relevant mechanism and machinery. Our recent cloning of mammalian orthologues of a yeast regulator of nonsense transcript stability may allow a mechanistic approach to the study of these observational phenomena (Perlick et al. 1996).

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0002-9297/97/6003-0030$02.00

Am. J. Hum. Genet. 60:730–732, 1997

CAG Repeat Expansions in Bipolar and Unipolar Disorders

To the Editor:

Family, twin, and adoption studies consistently have indicated that the familial aggregation of bipolar (BP) disorder and unipolar recurrent major depression (UPR) is accounted for largely by genetic factors. However, the mode of inheritance is complex. One of the possible explanations could be that a gene with variable penetrance and variable expression is involved (Hall 1990). Recently there have been reports on a new class of genetic diseases caused by an abnormal trinucleotide-repeat expansion (TRE) (Willems 1994; Lindblad and Schalling 1996). In a number of genetic disorders, these dynamic mutations were proved to be the biological basis for the clinically observed phenomenon of anticipation (Petronis and Kennedy 1995). DNA consisting of repeated triplets of nucleotides becomes unstable and increases in size over generations within families, giving rise to an increased severity and/or an earlier onset of the disorder (Morrison 1996). It has been recognized for a long time that anticipation occurs in multiplex families transmitting mental illness (Mott 1910). More recent studies also suggest that both BP disorder and UPR show features that are compatible with anticipation (McInnis et al. 1993; Nylander et al. 1994; Engström et al. 1995). Although the findings of anticipation in BP disorders and in UPR must be interpreted with caution because of the possible presence of numerous ascertainment biases (Penrose 1948), they support the hypothesis that pathological TREs are implicated in the transmission of these disorders. TRE combined with variable penetrance of expression could explain the complex transmission pattern observed in BP disorder. In view of this, the recent reports of an association between CAG-repeat length and BP disorder in a Belgian, Swedish, and British population are promising (Lindblad et al. 1995; O'Donovan et al. 1995).

In order to test the hypothesis that TREs underlie the genetic basis of BP and UPR disorders, we have analyzed the extent of CAG repeats in genomic DNA from a series of unrelated individuals of Croatian ancestry. Patients were recruited among in- or outpatients of the "Rebro" and "Vrapče" psychiatric hospitals in Zagreb, Croatia. They previously had been assessed for lifetime psychopathology by trained clinicians who used clinical interviews and hospital case notes. All patients were interviewed by one experienced psychiatrist (L.O.) using a standardized Croatian translation of the Schedule for Affective Disorders and Schizophrenia—Lifetime version (SADS-L) (Endicott and Spitzer 1978). All interviews were reviewed by a second experienced and senior psychiatrist (S.I.). Those who fulfilled research diagnostic criteria (RDC) for BP disorder type I (BPI) and for UPR were included. The patient population consisted of 42 individuals with BPI (25 females and 17 males; age range 31–70 years; mean age at onset 31.7 years ± 5.7 years) and 43 individuals suffering from UPR (34 females and 9 males; age range 30–74 years; mean age at onset 39.6 years ± 10.6 years). The age of the patient at the first contact with the treating psychiatrist was taken as the age at onset. Fifteen BPI and nine UPR patients had at least one first-degree relative with a major affective disorder as established through the family-history method. The information on family members was collected from patients but also was confirmed by reliable family members and, if available, by medical records. Control subjects with no personal or family history of psychiatric disorders (60 females and 21 males) were selected among the hospital staff and were matched for sex, age, and ethnic background.

The genomic DNA of patients and controls was analyzed by use of the $(CTG)_{10}$ oligonucleotide and the repeat expansion detection (RED) method (Schalling et al. 1993; Lindblad et al. 1995). The RED method, which allows testing for TRE in whole genomic DNA, has been described in detail elsewhere (Schalling et al. 1993). The